

The Examiner is encouraged to telephone the undersigned in order to expedite the prosecution of the instant application.

Respectfully submitted,
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MARKED-UP VERSION OF CLAIMS UNDER 37 C.F.R. §1.121(b)(1)

1. The use of an erythroid cell which is substantially undifferentiated, but which is capable of expressing a heterologous protein under the control of a globin promoter thereof, in an assay in which said protein interacts with an endogenous signalling cascade of said cell and said interaction is detected.

2. The use according to claim 1 wherein said erythroid cell is a murine erythroleukaemia (MEL) cell, rat erythroleukaemia cell (REL) or a human erythroleukaemia cell (HEL).

3. The use according to claim 2 wherein the erythroid cell is a murine erythroleukaemia cell.

4. The use according to ~~any one of claims 1 to 3~~ claim 1 wherein the said globin promoter is the β -globin promoter.

5. An ~~erythroid~~ erythroid cell which is substantially undifferentiated but which is capable of expressing proteins under the control of a globin promoter thereof at levels which allow use in accordance with ~~any one of claims 1 to 4~~ claim 1.

6. An erythroid cell according to claim 5 which comprises a cell as deposited at the European Collection of Cell Cultures under Accession number 99012801.

7. A method of producing an erythroid cell according to claim 5 which method comprises maintaining growing uninduced erythroid cells in culture for a sufficient period of time and isolating a subclone which expresses protein under the control of a globin promoter.

8. A method for determining the interaction between a receptor protein and a potential agonist or antagonist therefor, said method comprising

incubating a cell as defined above which has been transformed so that it expresses said receptor protein as a G-protein coupled receptor, either

(I) in (a) the presence and (b) the absence of said potential agonist; and/or

(II) in the presence of a known agonist and (a) the presence or (b) the absence of said potential antagonist; and monitoring and comparing G-protein coupled receptor induced signals in cells of (Ia) and (Ib) and/or (IIa) and (IIb).

9. A method according to claim 8 wherein the G-protein coupled receptor induced signal is monitored by measuring the calcium ion content of the cells.

10. A method according to claim 9 wherein the calcium levels are measured by means of a fluorescent indicator.

11. A method according to claim 8 wherein the G-protein coupled receptor induced signal results in a change in the cyclic AMP (cAMP) levels within the cell, and the G-protein induced signal is monitored by measuring the cyclic AMP content of the cells.

12. A method according to claim 11 wherein the cells are transformed with a reporter gene, expressed of which is regulated by a G-protein coupled receptor induced signalling cascade, and the G-protein coupled receptor induced signal is monitored by detecting the product of the reporter gene.

13. A method according to claim 12 wherein the reporter gene is β -GAL.

14. A method according to ~~any one of claims 8 to 13~~ claim 8 wherein the G-protein coupled receptor induced signal results in a decrease in the level of the measured cellular component, and tests (I) and (II) are carried out in the

presence of a chemical which contributes to an increased level of said cellular component.

15. A method according to claim 14 wherein the measured cellular component is cAMP and the said chemical is forskolin.

16. A method according to ~~any one of claims 7 to 15~~ claim 7 wherein the receptor is an insect receptor.

17. A method according to claim 16 wherein the insect receptor is a tyramine, a serotonin, a dopamine, an octopamine or a muscarinic-acetylcholine receptor.

18. A method according to ~~any one of claims 8 to 17~~ claim 8 wherein the cells are subsequently induced to differentiate, and used in a ligand binding assay.

19. An assay for detecting binding between a protein and a potential binding partner therefore, said method comprising (a) transforming a cell according to claim 5 so that the protein is expressed under the control of a globin promoter, (b) detecting binding between said potential binding partner and the said protein on a membrane of the cell.

20. An assay according to claim 19 where the cells are induced after step (a) and prior to step (b), so as to obtain high levels of protein expression from fully differentiated cells.

21. An assay according to claim 19 ~~or claim 20~~ wherein step (b) may be effected on isolated membranes extracted from lysed cells.

22. A vector comprising a sequence which encodes a non-mammalian protein receptor under the control of a globin promoter.

23. A vector according to claim 22 wherein the globin promoter is under the control of the human globin locus control region.

24. A vector according to claim 22 ~~or claim 23~~ wherein the non-mammalian protein receptor is an insect receptor.

25. A vector according to claim 24 wherein the insect receptor is the locust tyramine receptor.

26. A cell according to claim 5 ~~or claim 6~~ which is transformed with a vector according to any one of claims 22 to 25: comprising a sequence which encodes a non-mammalian protein receptor under the control of a globin promoter.

27. A cell according to claim 5 which has been transformed such that it contains a globin promoter associated with a cloning site and/or a reporter cassette containing a reporter gene, such as the β -galactosidase gene, under the control of a response element susceptible to modulation by a signalling cascade used in an assay.

28. A cell according to claim 26 which further comprises an enhancer, able to increase expression of a gene placed under the control of said globin promoter and/or is at an optimal distance of said reporter cassette such that the expression is dependent on the concentration of a particular downstream component in the signalling cascade.

29. A cell according to claim 28 wherein the enhancer is the LCR enhancer.